PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 4 : G01N 1/18, B01D 25/04

A1

(11) International Publication Number: WO 88/ 06723

(43) International Publication Date: 7 September 1988 (07.09.88)

(21) International Application Number: PCT/US88/00584

(22) International Filing Date: 24 February 1988 (24.02.88)

(31) Priority Application Number: 018,043

(32) Priority Date: 24 February 1987 (24.02.87)

(33) Priority Country: US

(71) Applicant: BIONIQUE LABORATORIES, INC. [US/US]; Bloomingdale Road, Saranac Lake, NY 12983 (US).

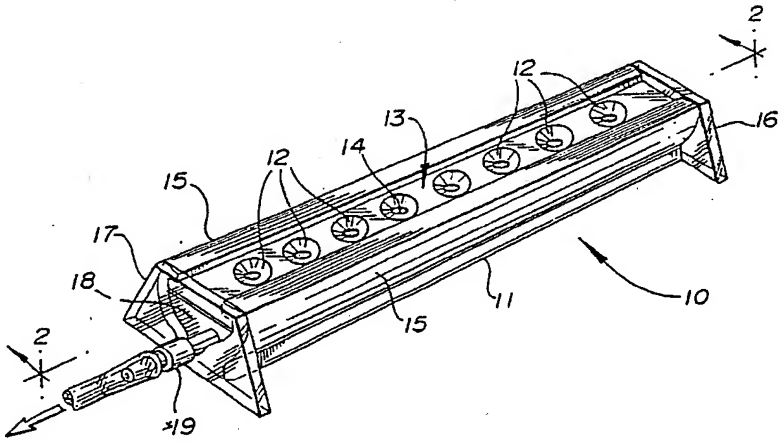
(72) Inventor: GABRIDGE, Michael, G. ; Trudeau Road, Saranac Lake, NY 12983 (US).

(74) Agent: ELMAN, Gerry, J.; Elman Associates, The Bourse Building, Suite 900, Philadelphia, PA 19106-2523 (US).

(81) Designated States: AT (European patent), BE (European patent), CH (European patent), DE (European patent), FR (European patent), GB (European patent), IT (European patent), JP, LU (European patent), NL (European patent), SE (European patent).

Published
With international search report.

(54) Title: DISPOSABLE IMMUNOASSAY AND BIOCHEMICAL TEST DEVICE SUITABLE FOR FIELD AND OFFICE USE



(57) Abstract

A disposable biochemical test apparatus for use in filter assays and static assays. A preferred form of the apparatus is substantially rectangular, comprising an upper surface (13), trapezoidal side support walls (15) providing stability, a bottom surface (11), and end plates (16, 17) at each end. The upper surface (13) forms a planar template containing six tapered cylindrical apertures or wells (12) arranged linearly and extending through the template. Underlying the template is a microporous membrane (14) bonded to the lower surface of the template. One of the end plates (17) has an exit port (19) which provides an exit for fluids from the enclosed region. The bottom surface (11) forms an exit ramp (18) slanting upward from the exit port (19) to the other end plate (16). The apparatus is provided as a single unit which may be disposed of after use. In use a syringe aspirates fluid by providing gentle vacuum through the exit port (19).

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	FR	France	ML	Mali
AU	Australia	GA	Gabon	MR	Mauritania
BB	Barbados	GB	United Kingdom	MW	Malawi
BE	Belgium	HU	Hungary	NL	Netherlands
BG	Bulgaria	IT	Italy	NO	Norway
BJ	Benin	JP	Japan	RO	Romania
BR	Brazil	KP	Democratic People's Republic of Korea	SD	Sudan
CF	Central African Republic			SE	Sweden
CG	Congo	KR	Republic of Korea	SN	Senegal
CH	Switzerland	LI	Liechtenstein	SU	Soviet Union
CM	Cameroon	LK	Sri Lanka	TD	Chad
DE	Germany, Federal Republic of	LU	Luxembourg	TG	Togo
DK	Denmark	MC	Monaco	US	United States of America
FI	Finland	MG	Madagascar		

DISPOSABLE IMMUNOASSAY AND BIOCHEMICAL TEST DEVICE SUITABLE FOR FIELD AND OFFICE USE

BACKGROUND OF THE INVENTION

1. Field of the Invention.

The present invention relates to an apparatus for biochemical testing, such as spot tests, immunoassays, and similar reaction sequence which may involve a colored or chromogenic end point. In particular, this invention relates to a multi-well device in which the lower surface of each well is a microporous membrane. Removal of reagents deposited in the wells is accomplished by applying a gentle vacuum across an enclosed region under the membrane with a syringe or equivalent device.

2. Background Information.

Microtiter wells are used in biochemical, clinical and biological laboratories for a multitude of functions including immunoassays such as the RadioImmunoAssay (RIA) and Enzyme Linked ImmunoSorbent Assay (ELISA). In the ELISA process, a critical specific reagent is tagged with an enzyme, and addition of the appropriate enzyme substrate results in the development of a visually detected chromogenic product.

Such assays are widely used to detect and quantify specific antibodies in serum. The antibody test sample is added to a microwell in a plastic plate previously coated with an antigen to which the antibody will attach in a specific fashion. Conversely, an antibody can be affixed to the wells to detect an antigen in test samples. To facilitate rinsing, many devices incorporate a microporous membrane in the lower well surface, such that liquid can pass through the well itself.

One of the known designs is a two-piece ELISA filter device using multiple wells and membranes which are precoated with antigens or antibodies. Therefore, use of this device is restricted to particular ligands. The membranes are

provided for regulation of fluid flow-through. Rather, the rate of fluid flow is governed by the hydrostatic pressure exerted on a drop of liquid deposited on the upper membrane surface as it rests on the top of the membrane. Reaction time is controlled by the resident time of the sample within the membrane pores.

Another example of a known device used to assay liquid samples for the presence of a diagnostic reagent consists of a telescoping top and bottom member which define a liquid reservoir therebetween. The top member has a multiplicity of test wells each having a bottom opening to the periphery to which the top member has a coreactant immobilized on its internal and external surfaces and permits passage of fluid through predetermined passageways. A sorbent material is placed in the reservoir between the two members. After the liquid sample is placed in a fluid passageway on the top member, the top member is depressed to pass the sample through the membrane into the sorbent material. The sorbent material is held in the base which consists of a surface layer preferably non-wetted by the liquids used and a bulk layer sufficiently thick to absorb all the liquids passing through the fluid passageways.

Still another apparatus is a reusable device consisting of a filter membrane clamped between a multi-well manifold and a vacuum chamber utilizing a sealing rubber gasket. A minute hole in the well bottom allows capillary action to hold the ligand above the filter until a standard vacuum is applied to the vacuum nipple using a connecting hose. The membrane may consist of an individual disk in each well, allowing removal of the individual disks, or a continuous sheet clamped between the upper element and its base.

Another device in use consists of an apparatus having a plurality of racks carrying cylindrical reaction vessels, each having a porous bottom connected to separate chambers. The reaction vessels can be emptied simultaneously by suction from a combination vacuum pump and pressure regulator.

The structures currently known are characterized by uneven and unreliable sealability between wells, resulting in well-to- well contamination. The lateral leakage is a serious failing because it obscures test results. The complexity of previous devices may require lengthy assembly time, frequently involving gaskets and clamps and/or screws to obtain a seal. Filter misalignment and improper assembly lead to air leaks and contamination of wells by lateral migration and regulators as a means to withdraw fluids from reaction wells are expensive, bulky, require power to run and make fine control of pressure very difficult. These characteristics make many of the known unsuitable for field use. Furthermore, the person performing the tests may be in contact with hazardous chemical solutions when the reservoirs are emptied and cleaned.

DISCLOSURE OF INVENTION

The present invention provides a disposable biochemical test apparatus for use in chromogenic filter-based assays and static assays. The apparatus comprises an upper surface, a side wall and a bottom surface to form an enclosed region. The upper surface comprises a planar template having top and lower surfaces and containing a plurality of spaced apertures extending from the top surface through the template to the lower surface, each aperture being larger in the top surface and smaller in the lower surface, thereby providing conical sloping sides in the aperture. A microporous membrane is placed in the enclosed region so as to underlie the template, the membrane having sufficient dimension to cover the lower apertures. An exit port is provided to enable fluids to exit the enclosed region. An exit ramp, covering the bottom surface and slanting upward from the exit port, is also provided.

The one-piece disposable test apparatus of the present invention is designed to provide a plurality of discrete wells arranged in a horizontal array to permit a multitude of biochemical tests to be run simultaneously. The device

overcomes the disadvantages of similar devices previously known by its simplicity of design and ease of operation. The device generally is comprised of a clear plastic block having a plurality of wells in its upper surface with the wells sloping inwardly. The sloping nature of the upper wells allows one to view the entire array of well bottoms and make visual or instrumental observations with ease.

The well bottoms are covered by a porous cellulose nitrate membrane, for example, and the lower surface of the enclosed region is ramped to an exit port. In other words, the exit ramp commences near the lower apertures and slants downward toward the exit port to enhance the migration of liquids to the exit port. A vacuum is created in the enclosed region to move liquids in the wells through the membrane toward the exit port of the apparatus. Flow through the wells is under operator control. All reactants and wash fluids are moved through the exit port to a reservoir and the entire assembly is disposed of without exposing the operator to any of the chemicals contained therein. The invention is designed to be simple, inexpensive to manufacture and may be comprised of two or more molded plates sealed together resulting in a one-piece disposable unit. Without any necessity for a vacuum pump and line current, the unit is especially well adapted to office and field use.

BRIEF DESCRIPTION OF DRAWINGS

Fig. 1 is a perspective view of one embodiment of the present invention;

Fig. 2 is a cross-sectional view taken along line 2--2 of Fig. 1;

Fig. 3 is an end view of the embodiment of Fig. 1;

Fig. 4 is a perspective view of another embodiment of the present invention;

Fig. 5 is a cross-sectional view taken along line 5--5 of Fig. 4;

Fig. 6 is a perspective view of a futher embodiment of the present invention; and

Fig. 7 is a cross-sectional view taken along line 7--7 of Fig. 6.

MODES FOR CARRYING OUT THE INVENTION

Fig. 1 depicts a disposable biochemical test apparatus 10 having an upper surface 13 containing apertures or wells 12 permitting fluids to contact a membrane 14 and pass through the membrane 14 into an enclosed region provided by the side walls 15, the upper surface 13, the bottom surface 11 and the end plates 16 and 17. The exit ramp 18 permits the liquid to migrate toward the exit port 19.

Fig. 2 shows the test apparatus of Fig. 1 in a cross-sectional view taken along line 2--2. The upper surface 13 contains apertures 12 which are tapered conical apertures with the smaller aperture at the bottom contacting a microporous membrane 14. After solutions are placed in the wells 12, and a vacuum is applied, the liquid passes through the membrane 14 and is deposited on the exit ramp 18. The liquids thereby exit at the exit port 19 into a syringe or like instrument which has provided suction and vacuum to draw the liquids through the membrane 14, the enclosed region 20, and exit port 19 in succession. The bottom surface 11, the end plates 16 and 17, and the side walls 15 along with the top surface 13 provide the enclosed region 20. The test apparatus and the syringe or like instrument containing the withdrawn liquid are disposed of after the results are recorded.

Fig. 3 is an end view through a clear plastic end plate 16. The upper surface 13 contains the apertures or wells 12 which exit onto a microporous membrane 14. The side walls 15, the bottom 11, the top surface 13 and the end plates as depicted by 16, provide the enclosed region 20 containing any liquids which may have migrated through the membrane within the interior of the structure.

Fig. 4 is a perspective view of a test apparatus 40 having an upper surface 43 in the shape of a frustum of a cone, in this case a circle, which has apertures or wells 42,

a side wall 45, a bottom surface 41 and an exit port 49.

Fig. 5 is a cross-sectional view taken along line 5--5 of Fig. 4. The lower portions of apertures 42 contact a membrane 46. The upper surface 43, the side wall 45, and the bottom surface 41 provide an enclosed region 44. A funnel-shaped exit ramp 48 is provided to assist in migration of liquids from the membrane 46 toward the exit port 49.

Fig. 6 is a perspective view of a test apparatus 60 which contains within it an integral plunger 68 and a reservoir 67 and 72 which enables use without the necessity of an external syringe or like instrument. As shown in Fig. 7, the apparatus 60 has apertures 62 which are covered on the bottom by a membrane 63. An exit ramp 65 lies below the membrane 63 and forms the lower portion of an enclosed region 64 such that liquids passing through the membrane 63 and entering the enclosed region 64 collect via gravity and drain through the lowermost port toward exit port 66, which is continuous with a reservoir 67. Within the reservoir 67 there is a tight fitting moveable plunger 68. By drawing back on the flange 71, which is connected to a shaft 69, the ratio of space in reservoir 67 to the space in reservoir 72 increases, thereby creating a partial vacuum in contiguous spaces 65, 66 and 67. The use of a plunger 68 affords control by the operator over the rate of passage of liquid through the membrane 63. A shaft guide 70 helps align the plunger-shaft 69 as it draws back the plunger 68. Alternatively, the fixed shaft guide 70 could be threaded, such that the shaft 69 and the plunger 68 are withdrawn by a circular motion of the flange 71 instead of a direct outward pull on the flange 71.

A typical embodiment of the present invention is a test apparatus containing eight wells spaced evenly apart in the top surface of a test plate, which test plate is a rectangular block approximately 2.5 cm by 14 cm by 1.5 cm high. The substantially rectangular block is made of a clear rigid inert plastic such as Lexan to facilitate the viewing of the fluid motion and transfer. The ends of the test

apparatus are plates which are trapezoidal in shape, having the long side of the trapezoid at the base for stability of the apparatus when it is placed on a surface for use.

The shape of the apparatus may vary and thus need not be rectangular. A circular embodiment is shown in Figs. 4 and 5. Other shapes such as a square or a triangle are satisfactory.

The microporous membrane may be constructed of any media capable of immobilizing a biochemical species including antigens, antibodies, cells, precipitates and the like. The pores are smaller than the particles one wishes to retain on the upper surface and also small enough so as to prevent the migration of liquid through the membrane by gravity alone. The pores are large enough, however, so that liquid will drain through the membrane when a gentle vacuum is applied. Suitable materials for the membrane include cellulose acetate, cellulose nitrate, mixed cellulose esters, nitrocellulose, nylon, polytetrafluoroethylene, polyethylene, and polypropylene. Other suitable materials will be apparent to those skilled in the art. In a preferred embodiment of the present invention, the membrane has pores averaging 0.45 microns in size.

The membranes can be affixed to the template containing the apertures or wells in any one of several ways. Bonding methods include chemical bonding, for example with a solvent or adhesive, thermal bonding, for example, ultrasonic welding, or the membrane may be molded in place. The precise method is inmaterial so long as lateral migration of liquid in the membrane is prohibited.

The apertures may be arranged in any desirable configuration so long as the apertures are spaced, preferably spaced at least 1 cm from center-to-center from each other. The size of the aperture as measured across the upper aperture is preferably about 7.5 mm in diameter; however, the size may vary according to need.

INDUSTRIAL APPLICABILITY

The lower surface of each well or aperture empties through the microporous membrane onto a sloped ramp which slopes toward an exit port to facilitate drainage. A vacuum is applied to the exit port to allow the removal of the accumulated fluids. Typically a 5- or 10-milliliter disposable syringe connected by a 5- to 13-cm length of inert plastic tubing facilitates fluid removal and allows fine control of fluid flow-through, i.e., pressure, to be easily accomplished. The flow-through mode of operation is useful in procedures involving contacts between mobile and immobile species wherein the latter is either covalently bound to the membrane or is unable to penetrate it and the former is suspended in the fluid in the well of the membrane.

The static mode of operation initially uses no vacuum and is useful in procedures requiring prolonged contact of two or more reactants in the fluid phase. Once the reaction is complete, vacuum is applied and filtration is achieved by utilizing the flow-through operation.

In a typical use of the invention, a test apparatus is connected through its exit port to a plastic disposable 5- or 10-milliliter syringe with a 7.5-cm length of tubing. Samples from 50- to 250-microliter aliquots are placed in each of the apertures or wells. The samples may be incubated for 5 to 10 minutes at 20 to 37 degrees C followed by removal through gentle vacuum created by pulling the syringe plunger outwardly. The test antigen may be a bacterial suspension or a soluble antigen bound to inert carrier particles.

Next a blocking agent is placed in the apertures. It may consist, for example, of 1% casein or 5% serum. Next is the addition in the apertures of a primary specific antibody. After the antibody is added the samples are treated with buffer rinse. In each of the steps vacuum is applies. Next a secondary antibody with a marker such as an enzyme, is added and is followed by a buffer rinse. Then an enzyme substrate is added which also is followed by a buffer rinse.

After each step, the plunger of the syringe is pulled outwardly just enough to cause the liquid in the wells to be pulled through the membrane and deposited in the enclosed region or in the reservoir of the syringe. At this point the test is completed and scores for the presence or absence of chromogenic or colored end products may be recorded by observation.

The volume of the enclosed region should be selected so that the application of vacuum supplied by a 5- or 10-milliliter syringe is sufficient to draw the fluids from the wells through the membranes and into the enclosed region. Generally, the capacity of the syringe is sufficient to pull the liquids into the enclosed region after each step in the process. In an apparatus wherein the syringe is part of the apparatus (see Figs. 6 and 7), the reservoir will be of sufficient size to apply vacuum after each step in the process. Thus the flange is pulled outwardly a little at a time until the procedure is completed thereby drawing any liquids residing in the wells through the membranes into the enclosed region and on into the reservoir.

The foregoing description is offered primarily for illustration purposes only. It is not intended that the present invention be limited to the particular structures and methods of operations set forth above. It will be readily apparent to those skilled in the art that numerous modifications and variations not mentioned herein can still be made without departing from the spirit and scope of the invention as herein claimed.

LISTING OF REFERENCE SIGNS AND FEATURES

test apparatus 10
bottom surface 11
apertures or wells 12
upper surface 13
membrane 14
side walls 15
end plate 16
end plate 17
exit ramp 18
exit port 19
enclosed region 20
circular test apparatus 40
bottom surface 41
apertures or wells 42
upper surface 43
enclosed region 44
side wall 45
membrane 46
exit ramp 48
exit port 49
test apparatus 60
apertures 62
membrane 63
enclosed region 64
exit ramp 65
exit port 66
reservoir 67
plunger 68
shaft 69
shaft guide 70
flange 71
reservoir 72

CLAIMS

Having thus described the invention, what it is desired to claim and thereby protect by Letters Patent is:

1. Disposable biochemical test apparatus for use in chromogenic filter assays and static assays under field and office conditions, said apparatus comprising an upper surface, a side wall and a bottom surface to form an enclosed region, said upper surface comprising a planar template having a top surface and a lower surface and containing a plurality of spaced apertures extending from the top surface through the template to the lower surface, each aperture being larger in the top surface and smaller in the lower surface thereby providing conical sloping sides in the apertures whereby the entire array of well bottoms may be viewed with ease; a microporous membrane in the enclosed region bonded to said lower surface so as to underlie the template, said membrane having sufficient dimension to cover the lower surface of the template and having pores that are smaller than the particles to be retained on the upper surface of the membrane and that are small enough to prevent the migration of liquid therethrough by gravity alone; an exit port located adjacent the lowest point of said bottom surface for the removal of fluids from said enclosed region; and an exit ramp located below said membrane and sloping downward toward said exit port.

2. The apparatus of Claim 1, wherein said membrane comprises a member selected from the group consisting of cellulose acetate, cellulose nitrate, a mixed cellulose ester, nitrocellulose, nylon, polytetrafluoroethylene, polyethylene, and polypropylene.

3. The apparatus of Claim 1, wherein said apertures are arranged with center-to-center spacing of at least about 1 cm and each aperture has a diameter of about 7.5 mm.

4. The apparatus of Claim 1 wherein said microporous membrane contains pores averaging about 0.45 microns in size.

5. The apparatus of Claim 1 wherein the shape of said apparatus is substantially rectangular.

6. The apparatus of Claim 5, wherein said side wall comprises a trapezoidal element having a base edge for resting on a horizontal work surface, wherein said base edge of the trapezoidal element is longer than the top edge of said element, whereby the apparatus is stabilized against inadvertently being tipped over.

7. The apparatus of Claim 1, wherein the shape of said apparatus is substantially that of a frustum of a cone and said exit ramp is substantially funnel-shaped.

8. The apparatus of Claim 1, wherein said exit port is attached by tubing to a syringe.

9. The apparatus of Claim 1, wherein said apparatus is provided with an integral reservoir containing a plunger such that when said plunger is activated, vacuum is created in said enclosed region and said reservoir.

FIG-1

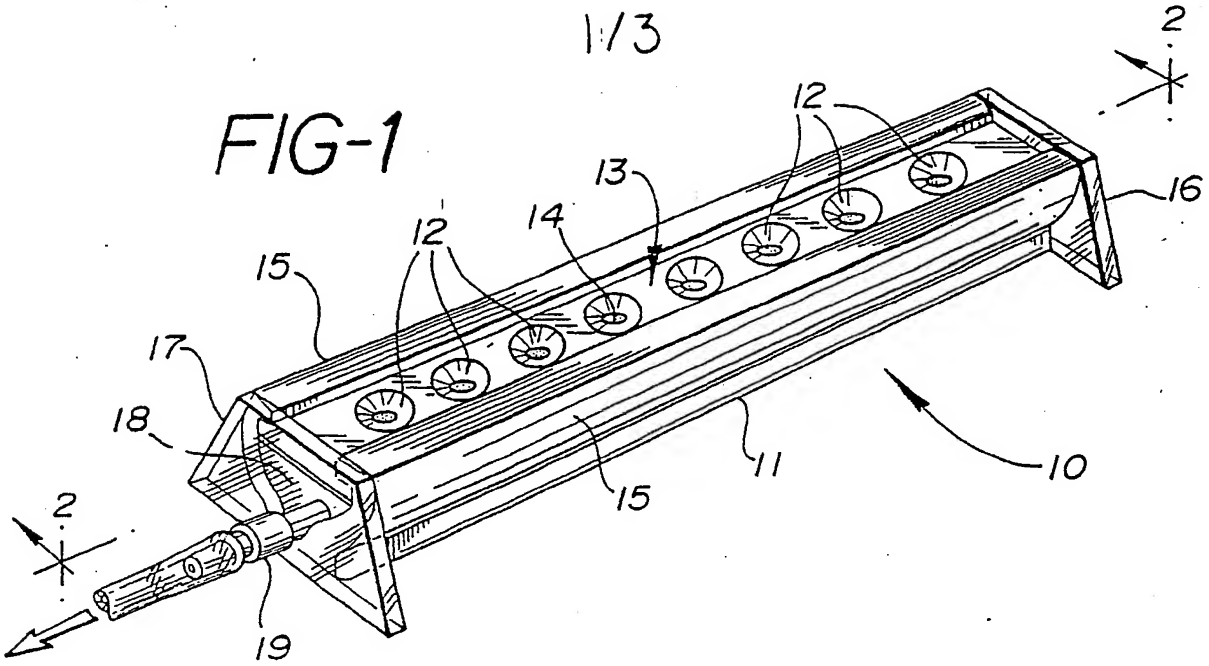


FIG-2

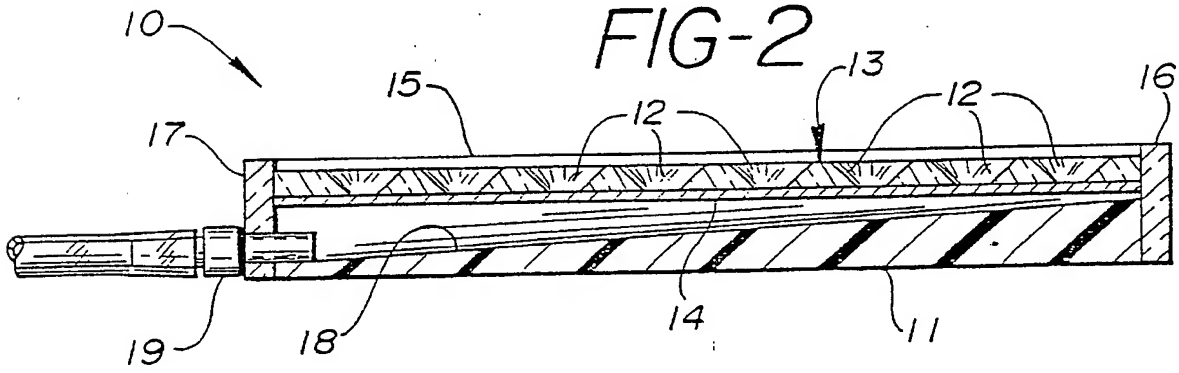


FIG-3

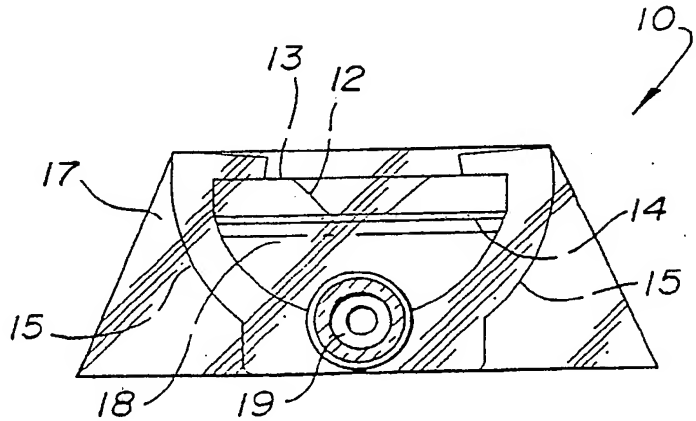


FIG-4

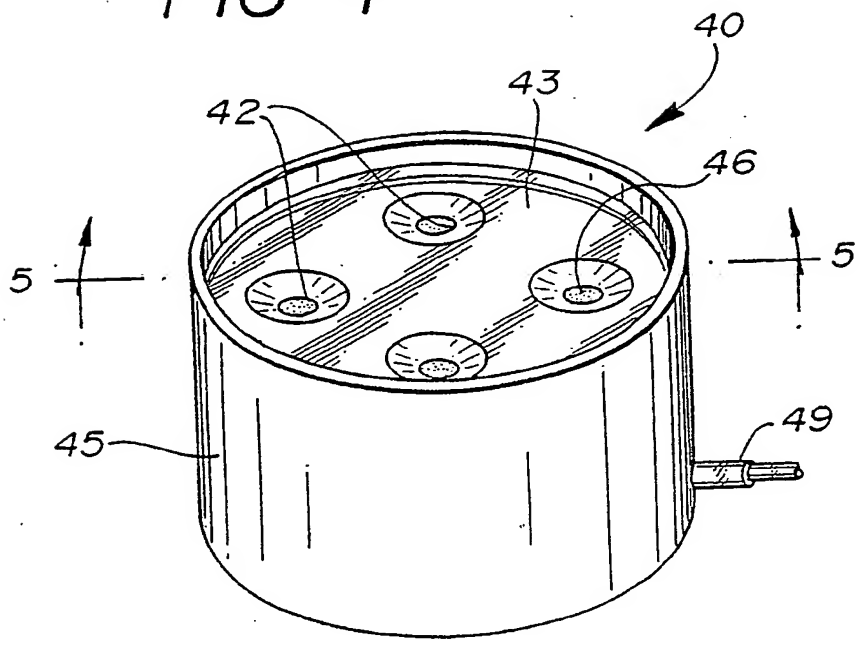


FIG-5

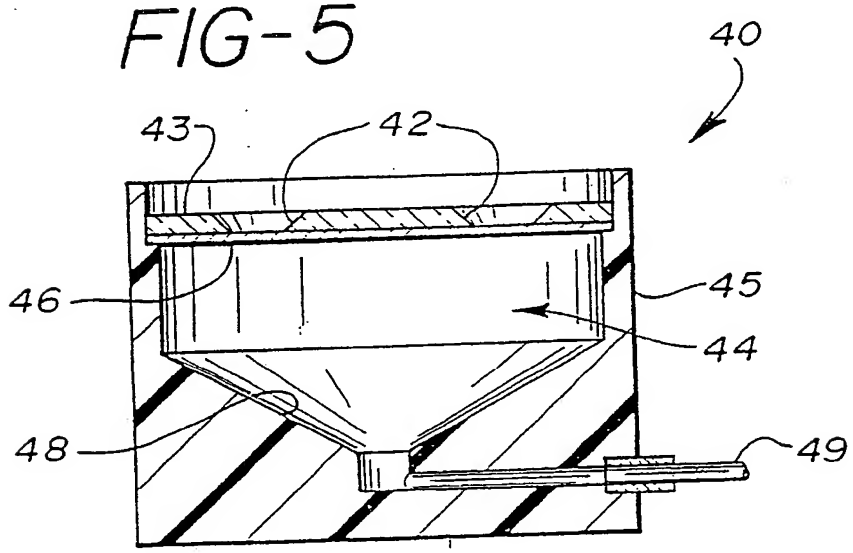


FIG-6

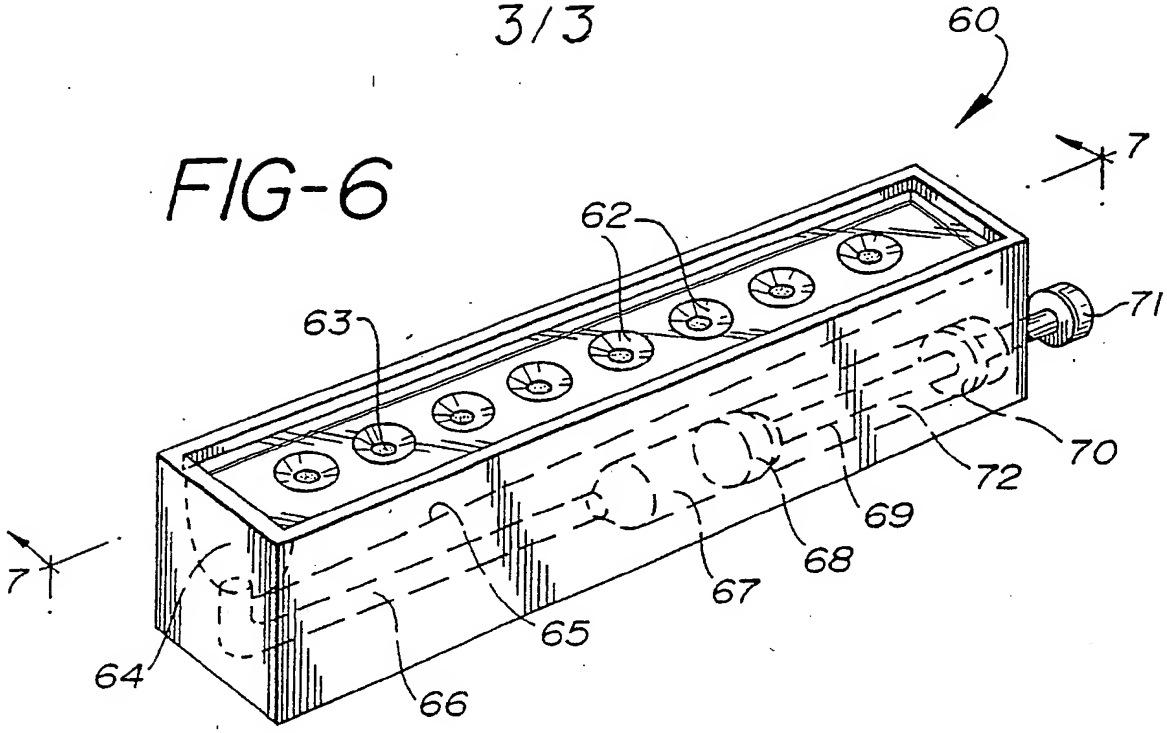
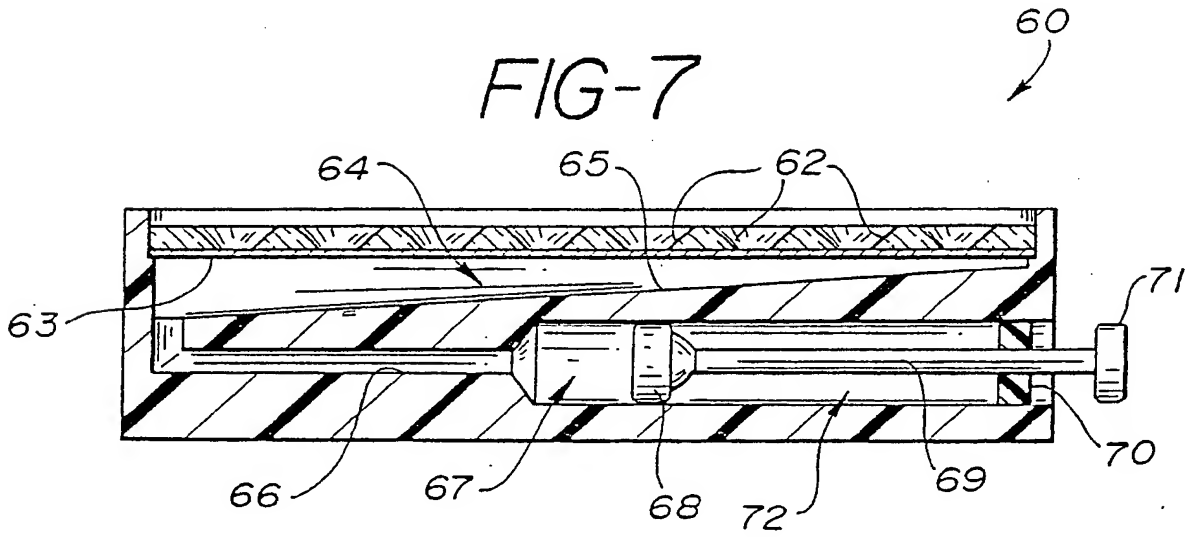


FIG-7



INTERNATIONAL SEARCH REPORT

International Application No PCT/US 88/00584

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [3]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC (4) : G01N 1/18; B01D 25/04
U.S. CL : 422/58, 99, 101, 102; 436/178, 809

II. FIELDS SEARCHED

Minimum Documentation Searched [4]

Classification System	Classification Symbols
U.S.	422/58, 61, 99, 101, 102; 436/177, 178, 809

Documentation Searched other than Minimum Documentation to the Extent that such Documents are included in the Fields Searched [6]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [14]

Category *	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No. [18]
Y	US, A, 3,356,462, (COOKE ET AL) 5 December 1967. See the entire document.	3
Y	US, A, 3,554,704, (USHAKOFF) 12 January 1971. See entire document.	7
A	US, A, 4,052,163, (PATZNER) 4 October 1977.	1-9
X	US, A, 4,090,850, (CHEN ET AL) 23 May 1978. See entire document.	1-2, 5-6
Y	US,A, 4,427,415, (CLEVELAND, 24 January 1984. See entire document.	1-6, 8-9
Y	US, A, 4,493,815, (FERNWOOD ET AL), 15 January 1985. See entire document.	1-6, 8-9
X/Y	US, A, 4,526,690, (KIOVSKY ET AL) 2 July 1985. See entire document.	1-2,4-6,8/9

* Special categories of cited documents: [15]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search [2]	Date of Mailing of this International Search Report [3]
21 May 1988	23 JUN 1988
International Searching Authority [1]	Signature of Authorized Officer [20]
ISA/US	L. KUMMERT

Form PCT/ISA/210 (second sheet) (October 1981)